We prepared the kinesin motor domain (K355) mutant that has a single cysteine at neck linker region and His-tag at C-terminal. Subsequently the kinesin mutant was dimerized with photochromic bifunctional cross-linker, azobenzene dimaleimide (ABDM). And the photo-reversible regulation of the ATPase and motor activities of the kinesin dimer cross-linked with ABDM was studied.
We also tried to develop photo-responsive vesicle composed of photochromic molecules as a cargo for the photo-controlled kinesin. Diacyl glycerol was coupled with carboxypropyl-spiropyran to be phospholipid analogue using carbonyldiimidazole condensation reagent. The spiropyran moiety performs photo-reversible isomerization between hydrophobic spiro form and merocyanine zwitterion form upon visible light and ultraviolet light, respectively. Therefore, it is expected that the merocyanine form of the mimic phospholipid results in formation liposome like vesicle. The photo-reversible formation of the vesicle was studied using water-soluble fluorescent probe.

680-Pos Board B460
Study of Phospho-Regulation of a Mitotic Kinesin using a Directed Evolution Approach
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The *S. cerevisiae* Cin8 belongs to the kinsesin-5 sub-family of mitotic motor proteins. During mitosis, Cin8 orchestrates the mitotic spindle assembly and its elongation. Recent work from our laboratory indicated that phosphorylation of Cin8 by Cdk1 governs its localization to the mitotic spindle during mitosis. Here we tested the rigidity of phosphorylation sites in Cin8, and examined whether phosphorylation at newly created Cdk1 sites can mimic the known phospho-regulation or create new regulation. For this purpose, we generated phosphorylation-deficient mutant of Cin8 and introduced new Cdk1 sites by single amino acid replacement. This resulted in thirty-one novel Cdk1 phosphorylation sites. In part of the sites, partial and full Cdk1 consensus sites were created. Next we analyzed Cin8 localization to the spindle during anaphase. We found that only one novel Cdk1 phosphorylation site at position 276 is able to restore the original phospho-regulation of Cin8, and is located in high proximity to a native Cdk1 phosphorylation site (S277). Although several sites were created nearby, only this site exhibits localization pattern which is similar to WT-Cin8. This result suggests that phospho-regulation of Cin8 by Cdk1 at this region is rigid and highly dependent on the structural context. Several additional novel Cdk1 mutants exhibited new phenotypes, suggesting that there are regions in Cin8 where phsopho-regulation by Cdk1 is more flexible. These results imply that phospho-regulation of Cin8 is more elusive than previously anticipated and further study of its mechanism is required.

681-Pos Board B461
Transport by a Kinesin in the Presence of Magnetic Nanoparticles
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Superparamagnetic nanoparticles are used to influence the medium in which kinesin nanotransport occurs. Simulation results show that nanoparticles form chain-like structures aligned with the direction of applied external magnetic field. The strength of the links in these chains depends on the properties of the particles and on the intensity of the applied magnetic field. Therefore, altering the magnetic field can be used to dynamically control the loads kinesins have to overcome - analogous to modifying properties of the medium in which the transport takes place.
The components of the motor protein, namely its two heads, two neck linkers and a neck and a cargo linker, are considered to be linear elastic elements. The chemical reaction of ATP/ADP and the heads is modeled using Michaelis-Menten kinetics and the Arrhenius equation. The overall model is shown to successfully capture the hand-over-hand motion of kinesin. By simulating the transport of a cargo by kinesin through obstacles created by the magnetic nanoparticles, it is shown that the resisting force created by chains of magnetic nanoparticles affects the speed of kinesin transport.
However, characterizing the motion of a kinesin in the presence of many magnetic nanoparticles requires stochastic simulations at a variety of conditions. The required computational time is prohibitive. Hence, a generalized model is developed to estimate the force on the cargo without solving the full-order system dynamics every time. Finally, the motion of cargo under varying magnetic fields is studied. These results can be used to detect possible deficiencies in kinesin - microtubule interactions.

682-Pos Board B462
Kinetic Characterization of Rice Plant Specific Kinesin E11 using Fluorescent ATP Analogue
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Kinesin is an ATP-driven motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics, and signal transduction. Kinesin species derived from vertebrates have been well characterized. In contrast, plant specific kinesin have yet to be adequately characterized. We have previously demonstrated that some kinesins derived from *rice* plant have unique biochemical characteristic properties and structures.
In this study, we characterized *rice* plant specific kinesin E11 that belongs to the plant specific At1 subfamily in kinesin-7 family. E11 motor domain was expressed by *E. coli* expression system and purified with Co-chelate column in order to characterize biochemical and ATPase kinetic properties. The fluorescent ATP analogues, Mant-ATP was employed for the kinetic characterization. We have successfully observed significant FRET between Mant-ATP and intrinsic tryptophan (Trp23) residue in E11. The kinetic parameters of initial binding of Mant-ATP to E11 and release of Mant-ADP from E11 were analyzed by monitoring the FRET using stopped flow apparatus and compared with other *rice* kinesins and conventional kinesin. The results revealed that the initial binding of ATP to E11 and release of ADP are slower than those of other *rice* plant specific kinesin.

683-Pos Board B463
Photo-Control of Mitotic Kinesin Eg5 using Thiol Group Reactive Fulgimide Derivative
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It is believed that the loop L5 of kinesin is important region for motor function. Interestingly mitotic kinesin Eg5 has a several times longer L5 in comparison with other kinesins. It has been demonstrated that the L5 of Eg5 performed as a stabilizer for the Eg5-specific inhibitors (STLC, monastrol) complexes. Aim of our study is to control the function of Eg5 photo-reversibly using photochromic molecules incorporated into L5. Previously, we have prepared Eg5 mutants (E116C,E118C,T125C,W127C,D130C) which have a single cysteine residue in L5 in order to incorporate photochromic molecules. We also synthesized thiol reactive photochromic molecules 4-phenylazomaleinanil(PAM) and Iodoacetyl-spiropyran (IASP). PAM and IASP were incorporated into the mutants stoichiometrically. Some of the Eg5 mutants modified with PAM and IASP showed reversible alteration of ATPase activity upon ultraviolet (UV) and visible (VIS) light irradiations. In this study, we synthesized a novel thiol reactive photochromic molecules monoiodoacetylfluglide(IAFG). Fulgimide performs photoreversible isomerzation between non-polar opened-ring form and polar closed-ring form upon visible light and ultraviolet light. IAFG was incorporated into Eg5 mutant W127C stoichiometrically. Althought the modified Eg 5 mutant W127C-IAFG showed slightly decreased ATPase activity, the ATPase activity showed photoreversible alteration upon UV and visible light irradiations. Alteration in the ATPase activity of W127C-IAFG in the presence of STLC upon UV and VIS light irradiations was also examined.

684-Pos Board B464
Photo-Regulation of Kinesin Intramolecularly Crosslinked by Bifunctional Azobenzne Derivative at the Coiled-Coil Stalk Region
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Kinesin is an ATP driven dimeric motor protein carries cellular cargoes along microtubules. The stalk region of kinesin is responsible for dimerization with coiled-coil interaction. Formation of dimer is essential for kinesin to perform processive movement along the microtubules. Aim of this study is to control dimerization of kinesin by the reversible conformational change at the coild-coil stalk region using photochromic molecule resulting in photo-reversible regulation of motility. Azobenzene-dimaleimide (ABDM) is a bifunctional SH reactive photochromic crosslinker and its crosslinking span is altered by cis-trans photo-isomerization of azobenznene moiety upon ultraviolet and visible light irradiations. We have previously demonstrated that the two reactive cysteine residues SH1(707) and SH2(697) in α-helix of myosin which region is believed to have a energy transducing role, were cross-linked by